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INSULIN METABOLISM AND ITS EFFECT ON BLOOD ELECTROLYTES AND GLUCOSE IN THE TURKEY HEN

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Abstract—1. Insulin half-life (T1/2) was determined to be similar between egg-laying and non-laying turkey hens, averaging 7.5 vs 8.7 min, respectively.

2. Infused insulin lowered plasma glucose 25% in both groups although the time course of each response was different.

3. Circulating phosphorous decreased 30 min following insulin treatment and returned to preinjection concentrations at the end of sampling.

4. Insulin initiated immediate decreases in plasma calcium and magnesium.

5. It is evident that insulin is involved in electrolyte metabolism as well as glucose metabolism in birds.

INTRODUCTION

The role of insulin in intermediary metabolism in birds is unorthodox compared to that in mammals (Hazelwood, 1973, 1976). A possible confounding factor prior to the availability of avian insulin was the use of mammalian insulins both *in vivo* and *in vitro* to assess the biological actions of insulin in avian species. Early studies in which insulin was either infused or injected, induced minimal hypoglycemic responses (Langslow *et al.*, 1970) leading to the hypothesis that birds are "insulin-resistant". The same authors noted the insulin-release response to prolonged hyperglycemia or hyperaminoacidemia was found to be transient. A similar transient insulin response (15–30 min) was observed *in vitro* only after glucose concentrations exceeded 500 mg/100 ml in culture media (Hazelwood, 1973). Such unconventional findings led to speculation that insulin had an extremely short half-life in birds. However, Langslow (1976) demonstrated that in chickens the half-lives of both avian and mammalian insulins were similar to that reported in mammals.

Our laboratory has initiated several studies investigating endocrine–nutrient interrelationships in the growing turkey poult and female hen. In preliminary studies it was observed that when blood glucose was elevated to values exceeding 1000 mg/100 ml in the turkey hen, a small and brief insulin response was detected (unpublished). In addition, it has been consistently noted that laying hens have higher blood insulin levels than age-matched nonlayers. Our knowledge of the role of the pancreas and/or pancreatic hormones in the growth and development of the turkey is virtually nonexistent. The study described in this paper was undertaken to determine insulin metabolism in the laying and nonlaying turkey hen and possible effects on electrolyte metabolism.

MATERIALS AND METHODS

Three nonlaying (6 hr light) and 3 laying (14 hr light) Large White turkey hens were used in this study. Birds were 34 weeks of age, weighing 9.1 kg, with the layers in the fourth week of egg production. Free access to water and a standard laying ration containing 3% calcium was allowed. Feed was removed during the sampling procedure. Three to 4 days prior to the initiation of the study, hens were anesthetized with pentobarbital and an indwelling cannula placed in their jugular veins to allow remote sampling. Highly purified chicken insulin (50 IU/mg) was obtained from Litron Laboratories, Rochester, NY. Insulin (50 μg) was dissolved in 1 ml saline and injected into a wing vein at 0.25 IU/kg body wt. Blood samples were drawn from the jugular cannula at the following times: −15, −5, 0 (pre-infusion), at 1 min intervals for the first 10 min, and at 20, 30, 40, 50, 60, 75, 90, 105 and 120 min post-infusion. Insulin content of the blood was determined by an homologous radioimmunoassay for chicken insulin (McMurtry *et al.*, 1983). Plasma calcium and magnesium were determined by atomic absorption spectrophotometry following dilution of unknowns in 0.5% lanthanum. Plasma phosphorous was determined as previously described by Fisk and Subbarow (1925) and glucose by the glucose oxidase method (Hill and Kessler, 1961). All sample determinations were performed in duplicate. Hormone half-life (T 1/2) was calculated using the following formula:

$$T\ 1/2 = 0.3 \times \text{interval (min between samples} \times \log_{10} \frac{\text{plasma conc. sample 1}}{\text{plasma conc. sample 2}}.$$

Data were analyzed by least squares procedures using the GLM procedure of the Statistical Analysis System (SAS, 1979).

RESULTS AND DISCUSSION

Circulating blood levels of a hormone are known to be a function of secretion rate and its clearance from blood. Changes in blood hormone levels during different physiological states may be due to alterations in either one or a combination of these factors. For example, the metabolic clearance of prolactin is more rapid in lactating than nonlactating ewes (Davis and Borger, 1973). The mean half-life (T 1/2) of injected chicken insulin in this study was 8.65 min in the nonlaying turkey hens and 7.51 min in the laying hens (Table 1). These values were not statistically

Table 1. Metabolism of chicken insulin in the turkey hen

Parameter	Nonlaying	Laying
T-1/2 (min)	8.65 ± .54	7.51 ± 1.19

Mean ± SEM of three observations.

significant. A T 1/2 of 7–8 min for turkeys is much longer than the 4.5 min reported for the chicken (Langslow, 1976; Colca and Hazelwood, 1976). The difference in T 1/2 values is not due to the use of chicken insulin in these studies, as structurally, chicken and turkey insulins are identical (Weitzel *et al.*, 1972). The metabolism of bovine and chicken insulin are similar in the chicken (Langslow, 1976). A half-life value of 9 min for insulin in the dog has been reported (Hommel *et al.*, 1971). The greater insulin half-life in turkeys compared to that reported to occur in the chicken could reflect differences in the method of determining hormone half-life or actual species differences. In this study, it is apparent that metabolic clearance is not influenced by the laying status of the turkey.

The bolus injection of insulin elevated circulating insulin levels 75-fold to 85-fold within 1 min after infusion when the initial post-injection blood sample was taken (Fig. 1). In both groups, circulating levels had returned to preinfusion levels (0.30 ng/ml plasma) within 75 min post-infusion.

The time-course effect of insulin on plasma glucose concentrations are shown in Fig. 2. Glucose levels were moderately albeit significantly ($P < 0.05$) lowered (23%), at 40 min post-injection in the nonlaying hens and remained depressed up to 75 min after-insulin treatment. In the laying hen, the delay in the glucose depressing effect of insulin occurred 10 min later. Preinfusion glucose concentrations were similar in the two groups. Plasma glucose in this group was not significantly lowered until 50 min after infusion and had started to rebound to pretreatment levels by 75 min. Plasma glucose had returned to pretreatment levels in both groups by 90 min post treatment (Fig. 2). The responses of turkeys to insulin is very similar to that reported for the chicken (Hazelwood and Lorenz, 1959; Langslow *et al.*, 1970; Vives *et al.*, 1981; Danby *et al.*, 1982). The magnitude of the hypoglycemic response in this study cannot be compared with that reported in the studies cited above because of the varying sources of insulin and doses injected. However, our studies demonstrate that insulin at physiological doses does have a role in regulating plasma glucose concentrations in the turkey.

A recent report has shown that insulin may have a role in mineral metabolism in birds (Palmieri *et al.*, 1979). The authors have demonstrated that insulin, when injected intravenously in young chicks, elicits a significant hypercalcemia. Insulin has been shown to stimulate bone resorption in chick embryos (Puche *et al.*, 1973). Because of its (insulin) potential importance in the regulation of electrolyte homeostasis during eggshell formation, electrolyte concentrations were also monitored in this study.

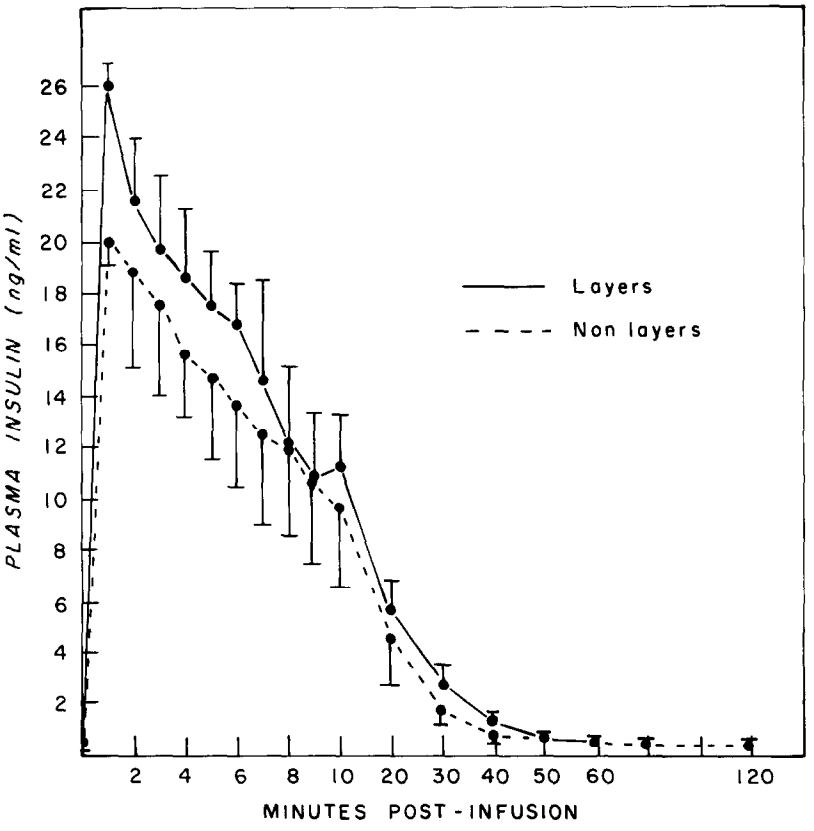

Fig. 1. Plasma insulin concentrations following the infusion of 50 μg chicken insulin in laying and nonlaying turkey hens. Values represent the mean ± SEM of three observations.

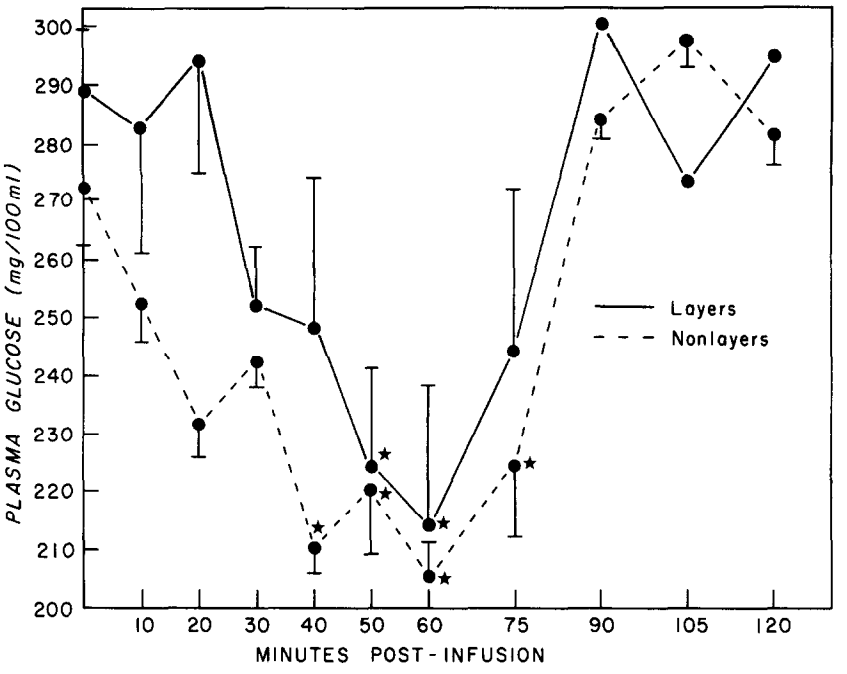

Fig. 2. Plasma glucose response to infused chicken insulin in the turkey hen. Preinfusion glucose values are the mean of the three samples taken prior to insulin treatment (−15, −5, and 0 min); ★ denotes that the mean is significantly different ($P < 0.05$) compared to preinfusion levels. When not shown, the standard error of the mean is smaller than the symbols. For clarity, plasma glucose concentrations at 1–9 min post-infusion are not shown.

The effect of insulin on blood phosphorous is illustrated in Fig. 3. Phosphorous levels were significantly greater ($P < 0.05$) at all sampling times in layers compared to nonlayers. In both groups, a significant drop in plasma phosphorous was noted 30 min following insulin treatment. At 50 min post-infusion, phosphorous had rebounded to preinfusion concentrations and remained elevated for the duration of sampling in the laying hen. A more gradual return to preinfusion levels was observed in the nonlayers. The response difference may reflect mechanisms (hormonal?) operating to protect the laying hen against chronic changes in blood electrolytes. The depressing effect of insulin on plasma phosphorous in turkey hens is similar to the significant hypophosphatemia reported to occur in

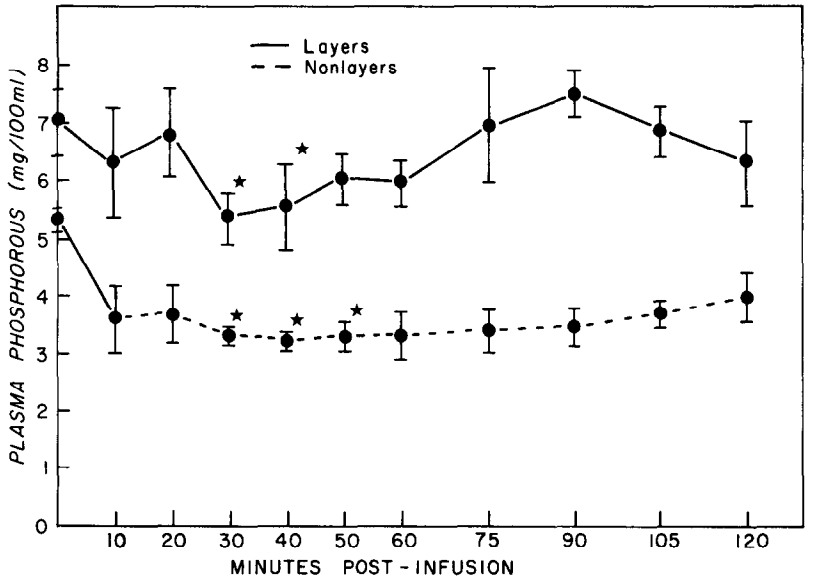

Fig. 3. Plasma phosphorous response to infused chicken insulin in the turkey hen. Preinfusion phosphorous values are the mean of the three samples taken prior to insulin treatment (−15, −5, and 0 min); ★ denotes that the mean is significantly different ($P < 0.05$) compared to preinfusion levels. For clarity, plasma phosphorous concentrations at 1–9 min post-infusion are not shown.

Table 2. Effect of insulin on plasma magnesium and calcium in the turkey hen

Sampling Time (minute)	Electrolyte			
	Mg^+ (mg/dl)		Ca^+ (mg/dl)	
	Layers	Nonlayers	Layers	Nonlayers
Preinfusion	3.95 ± .08	3.58 ± .06	27.09 ± .52	17.09 ± .52
Postinfusion				
1	3.80 ± .10	3.45 ± .15	26.10 ± .00	16.10 ± .00
2	3.63 ± .14	3.26 ± .06*	25.03 ± .86	15.03 ± .86
3	3.65 ± .25	3.36 ± .03*	24.90 ± .75	14.90 ± .75
4	3.47 ± .16*	3.33 ± .08*	24.97 ± .82	14.96 ± .82
5	3.55 ± .35	3.36 ± .14	24.15 ± .61*	14.15 ± .61*
6	3.40 ± .20*	3.36 ± .08*	23.35 ± .54*	13.35 ± .55*
7	3.36 ± .20*	3.36 ± .06*	25.50 ± .99	15.53 ± .99
8	3.70 ± .36	3.30 ± .11*	23.73 ± 1.16*	13.73 ± 1.17*
9	3.86 ± .29	3.33 ± .06*	24.10 ± 1.02	14.10 ± 1.02*
10	3.90 ± .32	3.33 ± .08*	26.23 ± 1.62	16.23 ± 1.62
20	3.70 ± .10	3.80 ± .15	26.75 ± .37	16.75 ± .37
30	3.65 ± .19	3.63 ± .08	25.10 ± .70	15.10 ± .70
40	3.60 ± .15	3.50 ± .15	25.27 ± .71	15.26 ± .71
50	3.46 ± .07*	3.23 ± .17*	25.10 ± .40	15.10 ± .40
60	3.50 ± .11	3.30 ± .05*	24.86 ± .63	14.86 ± .63
75	3.50 ± .15	3.46 ± .21*	25.30 ± .81	15.30 ± .81
90	3.36 ± .08*	3.66 ± .37	24.56 ± .33	14.56 ± .33
105	3.45 ± .15*	3.56 ± .17	24.70 ± .57	14.70 ± .57
120	3.33 ± .20*	3.56 ± .29	24.73 ± .53	14.73 ± .53

Values above represent the mean ± SEM of three observations. Means with an asterisk are significantly different ($P < 0.05$) than preinfusion levels. Preinfusion represents the mean of the three samples taken prior to insulin infusion (−15, −5, and 0 min).

young chicks with insulin at 60 min post-treatment (Palmieri *et al.*, 1979). It was noted that this was independent of a concurrent hypercalcemia. The differing time response to insulin (30 vs 60 min) in these two studies may reflect a difference in the type of bone present in the adult vs the young bird. Conversely, an increase in plasma phosphorous has been reported following 3 weeks of insulin treatment in chicks (Sood *et al.*, 1981).

An immediate and significant ($P < 0.05$) drop in circulating magnesium concentrations was noted in both layers and nonlayers following insulin treatment (Table 2). At 2 min post-infusion, plasma magnesium had declined 9% and fluctuated between 6 and 8% through 10 min post-infusion in nonlayers. Inexplicably, a significant drop was also noted at 50–75 min. Magnesium levels returned to preinfusion concentrations by the end of sampling. The insulin-induced decline in circulating magnesium was somewhat spurious in the laying hens (Table 2). Magnesium concentrations returned to near preinfusion levels very rapidly (within 5 min) following the initial drop noted at 4 min. Unlike the nonlayers, levels were suppressed beginning at $1\frac{1}{2}$–2 hr post-infusion. A relationship between insulin and blood magnesium concentration has not been previously reported.

Significant alterations in plasma total calcium were noted in both groups of hens following insulin treatment (Table 2). By 5 min, post-infusion blood calcium had significantly ($P < 0.05$) decreased (3 mg/dl) in both layers and nonlayers. Plasma calcium remained suppressed in both groups for approx. 4 min returning to pretreatment levels by 10 min. Although blood calcium never returned to preinfusion levels during the sampling period, this suppression was not significant. As expected, blood calcium was higher ($P < 0.05$) in the layers vs nonlayers.

The hypocalcemic effect of insulin observed in this study is opposite the hypercalcemic effect reported in chicks (10 days of age) (Palmieri *et al.*, 1979). The insulin-induced hypercalcemia has been shown to be due to a prompt effect of insulin on bone metabolism (Guthman *et al.*, 1982). Possible explanations for this difference are not readily apparent. It is noteworthy that the same authors and Simon (1980) reported no changes in plasma calcium in adult chickens following insulin or tolbutamide-induced insulin release. Differences could be due to age, species or sampling procedures employed in the various studies. Insulin is an important regulator of cartilage and bone growth in mammals (Canalis, 1983). In birds, in addition to its role in glucose metabolism, insulin may have an indirect role in calcium homeostasis. Clearly, more research is necessary to delineate the role of insulin (at physiological levels) on calcium metabolism in the adult bird.

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